COMBINATIONS OF A PYRIMIDINE CONTAINING NNRTI WITH RT INHIBITORS

Field of the Invention

The present invention concerns combinations of a pyrimidine containing NNRTI with nucleoside reverse transcriptase inhibitors and/or nucleotide reverse transcriptase inhibitors useful for the treatment of HIV infected patients or for the prevention of HIV transmission or infection.

Background of the Invention

Despite the fact that significant progress has been made by the introduction of HAART therapy (Highly Active Anti-Retroviral Therapy), resistance of the HIV virus against nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), protease inhibitors and even the more recent fusion inhibitors is still a major cause of therapy failure. For instance, half of the patients receiving anti-HIV combination therapy do not respond fully to the treatment, mainly because of resistance of the virus to one or more drugs used. Moreover, it has been shown that resistant virus is carried over to newly infected individuals, resulting in severely limited therapy options for these drug-naive patients. On the International AIDS Conference in Paris in July 2003, researchers released that the biggest study so far of resistance to AIDS drugs finds that about 10 percent of all newly infected people in Europe have drug-resistant strains. Smaller tests to determine the spread of resistance have been done in the high-risk city center of San Francisco. This test showed the highest level of resistance at 27 percent.

The pharmacokinetic profile of many commercially available antiretrovirals does not allow relatively low therapeutic doses. Poor pharmacokinetic profiles often in combination with poor solubility properties of the antiretrovirals cause the AIDS patient to face a high pill burden which is particularly undesirable for drug-naïve patients or first line therapy. Moreover, as a consequence of the AIDS virus even resisting antiretroviral combination therapy, a physician will boost the plasma levels of the active drugs in order for said antiretrovirals to regain effectivity against the mutated HIV viruses, the consequence of which is an even higher increase in pill burden. Boosting plasma levels may also lead to an increased risk of non-compliance with the prescribed therapy and to increased side-effects.

Several attempts have been made to date to design combination regimens. For instance,

the combination of lamivudine (a nucleoside RT inhibitor also named 3TC) at a 150 mg dose and zidovudine (a nucleotide RT inhibitor also named AZT) at a 300 mg dose, formulated in an oral tablet and dosed twice daily, or the combination of abacavir sulfate at a dose equivalent to 300 mg abacavir (a nucleoside RT inhibitor), lamivudine at a 150 mg dose and zidovudine at a 300 mg dose, formulated in an oral tablet and dosed twice daily.

WO 93/23021 describes therapeutic combinations for the treatment of HIV-infections comprising zidovudine and an agent serving to enhance the antiviral activity against HIV populations otherwise resistant to zidovudine.

WO 96/01110 describes a triple combination of zidovudine, lamivudine and loviride, the latter being a non-nucleoside RT inhibitor of the α-APA class.

An overview of new antiretroviral drugs is given in *Clinical Microbiology and Infection* 2003, Vol. 9 : 3, pp. 186-193.

WO 03/016306 specifically discloses more than 250 pyrimidine derivative having HIV replication inhibiting properties that act as non-nucleoside RT inhibitors (NNRTIs) having the ability to inhibit the replication both wild-type and of mutant strains. One of said NNRTIs is 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]amino]-benzonitrile (herein referred to as TMC278). WO 03/016306 also discloses the methods to synthesize these compounds. It further discloses combinations of said NNRTIs with other antiretrovirals, i.e. suramine, pentamidine, thymopentin, castanospermine, dextran (dextran sulfate), foscarnet-sodium (trisodium phosphono formate), zidovudine (3'-azido-3'-deoxythymidine, AZT), didanosine (2',3'-di-deoxyinosine; ddI), zalcitabine (dideoxycytidine, ddC), lamivudine (2'-3'-dideoxy-3'-thiacytidine, 3TC), stavudine (2',3'-didehydro-3'-deoxythymidine, d4T), abacavir, nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido-[3,2-b : 2',3'-e] [1,4]diazepin-6-one), efavirenz, delavirdine, TMC120, TMC125, tenofovir, (S)-8-chloro-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo-[4,5,1-jk] [1,4]benzodiazepine-2(1*H*)-thione, α-[(2-nitrophenyl)amino]-2,6-dichloro-benzene-acetamide, RO-5-3335, indinavir, ritonavir, saquinavir, lopinavir (ABT-378), nelfinavir, amprenavir, TMC126, BMS-232632, VX-175, T-20, T-1249, AMD-3100 and hydroxyurea.

Notwithstanding existing combination therapy, there is still a need for improved antiretroviral therapy, more particularly AIDS therapy. This need is particularly acute

for therapy that is effective not only on wild type HIV virus, but also on the increasingly more common resistant HIV viruses. It is thus highly desirable especially for first line therapy to design a combination regimen with a low pill burden that limits or even suppresses the recurrence of drug resistant virus and which can be used and remains effective for a long term.

It is an object of the invention to provide combinations of more than one therapeutically effective antiretroviral drug, which combinations can be used as first line therapy in drug-naïve patients for a long period of time.

It is also an object of the invention to provide combinations of more than one therapeutically effective antiretroviral drug in which the antiretroviral drugs have a complementary resistance profile thus creating a high resistance barrier and thus allowing a drug-naïve patient to take the combinations for a long period of time.

Another object of the invention is to provide combinations of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combinations can be administered once daily thus reducing the pill burden for the patient.

A further object of the invention is to provide combinations of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combinations can be co-formulated.

Yet a further object of the invention is to provide combinations of more that one therapeutically active antiretroviral drug wherein a therapeutically effective amount of each of the active antiretroviral drugs of the combinations can be co-formulated in one single pharmaceutical formulation.

Another object of the present invention is to provide combinations of more than one active antiretroviral drug which combinations can be used to prevent HIV transmission or infection in humans.

All references cited herein are incorporated by reference.

<u>Summary of the Invention</u>

Thus in a first aspect, the present invention provides a combination comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor and/or a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleotide

reverse transcriptase inhibitor and/or the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

Thus in a second aspect, the present invention provides a combination comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; wherein TMC278 and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In a third aspect there is provided a combination comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleotide reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In a fourth aspect there is provided a triple combination comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In a fifth aspect there is provided a triple combination comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a second nucleoside reverse transcriptase inhibitor different from the nucleoside reverse transcriptase inhibitor of (ii); wherein TMC278 and the first and second nucleoside reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another aspect there is provided a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and a combination as specified herein.

The invention also concerns the use of the combinations specified herein as HIV inhibitors and the use thereof in the treatment of HIV infected patients or in the prevention of HIV transmission or infection.

The invention is based on the finding that TMC278 is a potent reverse transcriptase

inhibitor that has an extremely high genetic barrier in combination with a favourable pharmacokinetic profile allowing once daily dosing. It was surprising to discover that TMC278 has all these properties together. This is unusual because one cannot predict what mutations will be selected in the HIV-1 genome by a given drug, whether the mutated virus will have any chance of survival under the pressure of the drug, how much drug is needed to limit or to suppress the recurrence of such mutated virus, and at what frequency such drug has to be given to maintain suppression of the development of a resistant virus that can break through the genetic barrier of the drug.

Detailed Description of the Invention

As used herein the term 'therapeutically effective HIV inhibitors at a dose that can be administered once daily' means that the HIV inhibitors are suitable for dosing every 24 hours. The 'term suitable for dosing every 24 hours' means that the HIV inhibitors are such that they can be administered every 24 hours and give effective blood plasma concentrations of the active ingredients such that they are effective to suppress HIV infection over a period of 24 hours. The HIV inhibitors for use in the invention can be dosed every 24 hours.

TMC278 or 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile is a known NNRTI, which can be prepared as described in WO03/016306. TMC278 can be used in base form or, which is preferred, as a suitable pharmaceutically acceptable salt form, in particular as an acid addition salt form. The pharmaceutically acceptable addition salts are meant to comprise the therapeutically active non-toxic salt forms. The acid addition salt forms can be obtained by treating the base form with appropriate acids as inorganic acids, for example, hydrohalic acids, e.g. hydrochloric, hydrobromic and the like; sulfuric acid; nitric acid; phosphoric acid and the like; or organic acids, for example, acetic, propanoic, hydroxyacetic, 2-hydroxy-propanoic, 2-oxopropanoic, oxalic, malonic, succinic, maleic, fumaric, malic, tartaric, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzene-sulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-2-hydroxybenzoic and the like acids. Preferred for use in the present invention are the hydrohalic acid salts, in particular the hydrochloride salt.

TMC278 occurs in stereoisomeric forms, more in particular as E- and Z-isomeric forms. Both isomers may be used in the combinations of the present invention. Whenever reference is made herein to TMC278, the E- and the Z-form as well as any mixture of both forms are meant to be included.

A preferred form of TMC278 for use in the invention is the E-isomer, i.e. (E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (hereinafter called E-TMC278). The Z-isomer of TMC278, i.e. (Z)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (hereinafter called compound Z-TMC278) can also be used. It has relatively high potency against wild-type HIV-1 but is less active against single and double mutants in comparison to the E-isomer. Table 1 shows the IC_{50} value in nM of the E and Z-isomer of TMC278.

Table 1

HIV RT mutation	E-isomer	Z-isomer
Wild-type	0.4	0.6
100I	0.4	6.3
103N	0.3	1.6
181C	1.3	5.0
188L	2.0	32
227C	2.0	4.0
100I+103N	7.9	790
103N+181C	1.0	40
227L+106A	1.0	4.0

Whenever reference is made herein to the E-form of TMC278 (i.e. E-TMC278), the pure E-isomer or any isomeric mixture of the E- and the Z-forms wherein the E- form is predominantly present is meant to be comprised, i.e. an isomeric mixture containing more than 50% or in particular more than 80% of the E-form, or even more than 90% of the E-form. Of particular interest is the E-form substantially free of the Z-form. Substantially free in this context refers to E-Z-mixtures with no or almost no Z-form, e.g. isomeric mixtures containing as much as 90%, in particular 95% or even 98% or 99% of the E-form. Equally, whenever reference is made herein to the Z-form of TMC278 (i.e. Z-TMC278), the pure Z-isomer or any isomeric mixture of the Z- and the E-forms wherein the Z-form is predominantly present is meant to be comprised, i.e. an isomeric mixture containing more than 50% or in particular more than 80% of the Z-form, or even more than 90% of the Z-form. Of particular interest is the Z-form substantially free of the E-form. Substantially free in this context refers to E-Z-mixtures with no or almost no E-form, e.g. isomeric mixtures containing as much as 90%, in particular 95% or even 98% or 99% of the Z-form.

Also meant to be included for use in this invention are salts of the isomeric forms of TMC278, in particular the salts mentioned above. Of particular interest are Z-TMC278 hydrochloride and specifically E-TMC278 hydrochloride.

Advantageously, the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to TMC278. Of particular interest therefore is any combination specified herein wherein (1) TMC278 and the nucleoside/nucleotide reverse transcriptase inhibitor or inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleoside/nucleotide reverse transcriptase inhibitor or inhibitors select mutations in the reverse transcriptase that do not cause resistance to TMC278.

Specifically, in one embodiment, a combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, wherein (1) TMC278 and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleoside reverse transcriptase inhibitor selects mutations in the reverse transcriptase that do not cause resistance to TMC278. In another embodiment, a combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleotide reverse transcriptase inhibitor, wherein (1) TMC278 and the nucleotide reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleotide reverse transcriptase inhibitor selects mutations in the reverse transcriptase that do not cause resistance to TMC278.

In a preferred embodiment, a triple combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a nucleotide reverse transcriptase inhibitor, wherein (1) TMC278 and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor select mutations in the reverse transcriptase that do not cause resistance to TMC278. In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) a second nucleoside reverse transcriptase inhibitor different from the nucleoside reverse transcriptase

inhibitor of (ii); wherein (1) TMC278 and the nucleoside reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleoside reverse transcriptase inhibitors select mutations in the reverse transcriptase that do not cause resistance to TMC278.

Preferred nucleotide reverse transcriptase inhibitors that can be used in the combinations subject of this invention include tenofovir and its prodrug tenofovir disoproxil fumarate.

Tenofovir is an adenosine nucleotide analogue currently commercially available with activity against retroviruses. Tenofovir disoproxil fumarate (tenofovir DF) is a once-daily, orally administered prodrug of tenofovir. For antiviral activity, tenofovir DF needs to be hydrolysed to the ANP analogue and then phosphorylated to the active diphosphate moiety [Arimilli et al *Antiviral Chemistry and Chemotherapy* 1997, 8 :6 (557-564); Fridland et al. *Antiviral Research* 1997, 34]. After entry in to lymphocytes or macrophages, the prodrug is quantitatively converted to the parent analogue, tenofovir, and phosphorylated to mono- and diphosphate metabolites. The cellular enzymes that are responsible for phosphorylation of this drug are adenylate kinase and nucleoside diphosphate kinase [Robbins et al. *Antimicrobial Agents and Chemotherapy* 1995, 39 :10 (2304-2308); Robbins et al. *Antimicrobial Agents and Chemotherapy* 1998, 42 :3 (612-617)]. Unlike other nucleoside analogues, such as zidovudine or stavudine, both of whose phosphorylation is cell cycle-dependent, tenofovir is efficiently phosphorylated in resting as well as cycling peripheral blood lymphocytes [Robbins et al. 1998]. Tenofovir can inhibit HIV-1 replication in different cell types that may target HIV, including primary human blood lymphocytes and macrophages [Perno et al. *Antiviral Research* 1992 (289-304); Perno et al. *Molecular Pharmacology* 1996, 50 :2 (359-366)]. The primary target of tenofovir diphosphate is reverse transcriptase (RT). Tenofovir diphosphate is a competitive inhibitor for the incorporation of deoxyadenosine triphosphate into nascent proviral DNA chains. Inhibition of HIV-1 RT by tenofovir diphosphate has an inhibition constant of approximately 0.9 μM, and if the analogue is incorporated into the growing viral DNA chain it may terminate further chain elongation. Tenofovir inhibits viral RT much more effectively than it inhibits cellular DNA polymerases [Suo et al *Journal of Biological Chemistry* 1998, 273 :42 (2750-2758)]. The concentration required to inhibit the replication of various HIV-1 strains by 50% (EC50) in lymphocyte and macrophage cell types (MT-2, CEM, ACH8) ranges from 0.2 to 10 μM. The antiviral effect is achieved at non-toxic doses of tenofovir (selectivity index ranging from 100 to 2000). Tenofovir DF is currently available as 300 mg tablets to be taken once daily.

Viral resistance to tenofovir in vitro emerges slowly. A recombinant virus expressing the K65R mutation showed a 3-fold decreased susceptibility to tenofovir in vitro [Cherrington et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1997, 37th]. Notably, clinical HIV strains expressing the M184V lamivudine-associated resistance mutation on RT show wild-type or increased susceptibility to tenofovir in vitro, independent of changes in Ki for the mutant enzyme [Miller et al. *Interscience Conference on Antimicrobial Agents and Chemotherapy* 1998,]. Long-term treatment (5 to 15 weeks) of newborn rhesus macaques with tenofovir (doses of 30 mg/kg) starting 3 weeks after inoculation with simian immunodeficiency virus, resulted in emergence of SIV with approximately 5-fold decreased susceptibility to tenofovir [Van Rompay et al. *Antimicrobial Agents and Chemotherapy* 1996, 40 :11 (2586-2591)]. This low level of resistance was associated with the appearance of the K65R mutation.

In a preferred embodiment, a combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) tenofovir or its prodrug tenofovir disoproxil fumarate, wherein TMC278 and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, and (iii) tenofovir disoproxil fumarate; wherein TMC278 and the nucleoside reverse transcriptase inhibitor and tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

Preferred nucleoside reverse transcriptase inhibitors that can be used in the combinations of this invention include abacavir or a pharmaceutically acceptable salt thereof, emtricitabine, racemic FTC and lamivudine (also named 3TC).

Emtricitabine or (-)-FTC is the left (-) rotatory enantiomeric form of racemic FTC or (±)-cis-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone (FTC). It is a commercially available nucleoside analogue and exhibits activity against HIV-1 [Hoong et al. *Journal of Organic Chemistry* 1992 (5563-5565); Jeong et al *Journal of Medicinal Chemistry* 1993, 36 :2 (181-195); Van Roey et al. *Antiviral Chemistry and Chemotherapy* 1993, 4 :6 (369-375]. The in vitro anti-HIV-1 activity of (-)-beta-enantiomer of FTC was reported to be 20-fold more than the (+)-

beta-enantiomer, and the (+)-enantiomer was significantly more toxic than the (-)-enantiomer to myeloid progenitor cells [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1992, 36 :11 (2423-2431)]. The potential for HIV-1 resistance to FTC was evaluated by serial passage of the virus in human PBMCs and MT-2 cells in the presence of increasing drug concentrations. Highly drug-resistant HIV-1 variants dominated the replicating virus population after two or more cycles of infection. RT derived from drug-resistant viral particles was 15- to 50-fold less susceptible to the 5'-triphosphate of FTC compared with the enzyme from parental drug susceptible virus. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met (ATG) to Val (GTG or GTA) [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1993, 37 :4 (875-881); Tisdale et al *Antiviral Research* 1993, 20 :Suppl 1; Smith et al *Journal of Virology* 1997, 71 :3 (2357-2362); Harrer et al *Journal of Infectious Diseases* 1996, 173 :2 (476-479); Tisdale et al *Proceedings of the National Academy of Sciences of the United States of America* 1993, 90 :12 (5653-5656)]. Due to this observed single mutation in the YMDD of reverse transcriptase in the HIV-infected patients, (-)-FTC is not suitable for monotherapy and needs to be administered in combination with other antiretroviral agents to effectively treat patients infected with HIV. Emtricitabine is available as 200 mg capsules to be taken once a day.

Lamivudine has the chemical name (-)-2',3'-dideoxy-3'-thiacytidine and is described for instance in EP-382 526 as an antiviral nucleoside analogue. It is also a well established and useful antiretroviral which is commercially available for instance as 150 mg oral tablets. Lamivudine is also commercially available in combination with zidovudine (300 mg zidovudine / 150 mg lamivudine), and in combination with lamivudine and abacavir sulfate (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

Abacavir is a well established and useful antiretroviral which is commercially available for instance as an oral solution of abacavir sulfate in a strength equivalent to 20 mg abacavir base/ml, or as an oral tablet of abacavir sulfate in a strength equivalent to 300 mg abacavir base. Abacavir sulfate is also commercially available in combination with lamivudine and zidovudine (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

Abacavir is a carbocyclic nucleoside with potent and selective anti-HIV activity. Abacavir in its optically active form is disclosed in EP-434 450. The cis-isomer of abacavir with unspecified absolute stereochemical configuration is described in

EP-349 242. Abacavir is one of the most potent NRTI developed to date. An average reduction in viral load of more than 1.4 log10 RNA copies/ml is observed after a short course of abacavir monotherapy. In vitro, resistant virus is not rapidly selected by abacavir. A significant decrease in susceptibility to abacavir in wild-type or zidovudine-resistant HIV-1 strains was not observed until after eight to ten passages in MT-4 cells. A set of resistance mutations at HIV reverse transcriptase (RT) codons, 65R, 74V, 115F and/or 184V, are selected during in vitro passage with abacavir, and a combination of these mutations was required to confer a 10-fold reduction in abacavir susceptibility in a laboratory strain of HIV. The first mutation detected upon passage of HIV-1 in an increasing concentration of abacavir is M184V, which confers only a 3-fold decrease in HIV-1 susceptibility. Phenotype resistance to 3TC and/or the presence of the 184V mutation does not prevent viral load response to abacavir therapy. Resistance to multiple nucleosides is associated with a decreased or absent response to abacavir [Kumar et al *Antimicrobial Agents and Chemotherapy* 1999, 43:3 (603-608); Lanier et al *International Conference on Retroviruses and Opportunistic Infections* 1998, 5th:Chicago; posted on 16 April 1999].

In a preferred embodiment, a combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof, and (ii) emtricitabine, wherein TMC278 and emtricitabine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In a preferred embodiment, a combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) lamivudine, wherein TMC278 and lamivudine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) abacavir or a pharmaceutically acceptable salt thereof, characterized in that, TMC278 and abacavir are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) abacavir sulfate, characterized in that, TMC278 and abacavir sulfate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) emtricitabine, and (iii) a nucleotide reverse transcriptase inhibitor, wherein TMC278 and the nucleotide reverse transcriptase inhibitor and emtricitabine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) lamivudine, and (iii) a nucleotide reverse transcriptase inhibitor, wherein TMC278 and the nucleotide reverse transcriptase inhibitor and lamivudine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) abacavir or a pharmaceutically acceptable salt thereof, or preferably abacavir sulfate, and (iii) a nucleotide reverse transcriptase inhibitor, wherein TMC278 and the nucleotide reverse transcriptase inhibitor and abacavir or a pharmaceutically acceptable salt thereof, or preferably abacavir sulphate, are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) emtricitabine, and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, wherein TMC278 and emtricitabine and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) lamivudine and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, wherein TMC278 and lamivudine and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In another preferred embodiment, a triple combination is provided comprising (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt

thereof; or a prodrug thereof; and (ii) abacavir or a pharmaceutically acceptable salt form thereof, preferably abacavir sulfate; and (iii) tenofovir or its prodrug tenofovir disoproxil fumarate, wherein TMC278 and abacavir or a pharmaceutically acceptable salt form thereof, preferably abacavir sulfate and tenofovir or its prodrug tenofovir disoproxil fumarate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

The following preferred triple combinations are also included

(a) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; with emtricitabine and tenofovir disoproxil fumarate;

(b) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; with lamivudine and tenofovir disoproxil fumarate.

(c) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; with abacavir sulfate and tenofovir disoproxil fumarate.

(d) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; with emtricitabine and lamivudine;

(e) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; emtricitabine and abacavir or a pharmaceutically acceptable salt thereof, preferably abacavir sulfate.

(f) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; abacavir or a pharmaceutically acceptable salt thereof, preferably abacavir sulfate and lamivudine.

In particular, in each of the combinations (a) – (f) the active ingredients, in particular TMC278, emtricitabine, lamivudine, abacavir or a pharmaceutically acceptable salt form thereof, preferably abacavir sulfate, and tenofovir or its prodrug tenofovir disoproxil fumarate, are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

The double combinations of the present invention may contain one or more additional active ingredients, which can be agents useful for treating HIV infected patients or other active agents. The triple combinations of the present invention may equally contain one or more additional active ingredients, which can be agents useful for treating HIV infected patients or other active agents. Preferably any of these additional agents are therapeutically effective at a dose that can be administered once daily.

The active ingredients of the combinations of the present invention may be administered simultaneously, concurrently or sequentially. Simultaneous

administration may be done by employing a unitary pharmaceutical formulation or separate pharmaceutical formulations. In general, the combinations may be administered by topical, oral, rectal, intravenous, subcutaneous or intramuscular routes. For first line therapy of HIV infection, simultaneous administration employing a unitary pharmaceutical formulation is preferred.

Thus, in another aspect there is provided a product containing a combination as specified herein as a combined preparation for simultaneous, separate or sequential use against HIV infection.

The invention also provides a product containing (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor and/or a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleotide reverse transcriptase inhibitor and/or the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily; as a combined preparation for simultaneous, separate or sequential use against HIV infection.

In a further aspect there is provided a product containing (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; wherein TMC278 and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily; as a combined preparation for simultaneous, separate or sequential use against HIV infection.

In another aspect there is provided a product containing (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleotide reverse transcriptase inhibitor, wherein TMC278 and the nucleotide reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily; as a combined preparation for simultaneous, separate or sequential use against HIV infection.

In another aspect there is provided a product containing (i) TMC278 or a stereo-isomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily; as a combined preparation for simultaneous, separate or sequential use against HIV infection.

In another aspect there is provided a product containing (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a second nucleoside reverse transcriptase inhibitor other than the nucleoside reverse transcriptase inhibitor of (ii); wherein TMC278 and the nucleoside reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily; as a combined preparation for simultaneous, separate or sequential use against HIV infection.

The active ingredients in the products of the invention are present in therapeutically effective amounts, the latter meaning an amount that is sufficient to exert a sufficient HIV inhibitory effect during a certain time period, i.e. the time period between each intake of the formulations, preferably for about 24 hours.

Particular embodiments are products as specified above containing one or more of the specific active ingredients mentioned herein such as emtricitabine, racemic FTC, lamivudin, tenofovir and its prodrug tenofovir disoproxil fumarate.

The products as mentioned above may contain separate formulations of the active ingredients, or two or where applicable more of the active ingredients may be co-formulated.

In still a further aspect the invention provides pharmaceutical formulations containing a combination as specified herein and a suitable carrier.

In another aspect there is provided a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and as active ingredients (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor and/or a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleoside reverse transcriptase inhibitor and/or the nucleotide reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

The invention further provides a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and as active ingredients (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; wherein TMC278 and the

nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In still another aspect there is provided a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and as active ingredients (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleotide reverse transcriptase inhibitor, wherein TMC278 and the nucleoside reverse transcriptase inhibitor and the nucleotide reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In still another aspect there is provided a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and as active ingredients (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a nucleotide reverse transcriptase inhibitor; wherein TMC278 and the nucleoside reverse transcriptase inhibitor and the nucleotide reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

In still another aspect there is provided a pharmaceutical formulation comprising a pharmaceutically acceptable carrier and as active ingredients (i) TMC278 or a stereoisomeric form thereof; or a pharmaceutically acceptable salt thereof; or a prodrug thereof; and (ii) a nucleoside reverse transcriptase inhibitor; and (iii) a second nucleoside reverse transcriptase inhibitor different from the nucleoside reverse transcriptase inhibitor of (ii); wherein TMC278 and the nucleoside reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

The active ingredients in the pharmaceutical formulations of the invention are present in therapeutically effective amounts, the latter meaning an amount that is sufficient to exert a sufficient HIV inhibitory effect during a certain time period, i.e. the time period between each intake of the formulations, preferably for about 24 hours.

Particular embodiments are pharmaceutical formulations as specified above containing one or more of the specific active ingredients mentioned herein such as emtricitabine, racemic FTC, lamivudin, tenofovir and its prodrug tenofovir disoproxil fumarate.

The pharmaceutical formulations of the present invention may be formulated into various forms for different types of administration. To prepare the pharmaceutical

formulations of this invention, effective amounts of the active ingredients, optionally in addition salt form, is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. The pharmaceutical formulations of the invention are preferably formulated in unitary dosage form suitable, particularly, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the formulations in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs, emulsions and solutions; or solid carriers such as starches, sugars, kaolin, diluents, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed.

In one aspect of the invention, the present combinations can be formulated in an oral tablet form further comprising pharmaceutically acceptable excipients having a weight ranging between 150 mg and 600 mg, suitable ranging between 200 and 400 mg. Convenient oral tablet forms containing the active ingredients according to the present invention have a total nominal weight ranging between 200 mg and 1500 mg, suitably between 500 mg and 1250 mg, more suitable between 600 and 1100 mg.

An advantage of the pharmaceutical formulations of the invention resides in the fact that each of the ingredients of the present combinations can be co-formulated in one pharmaceutical formulation and do not have to be administered separately. The daily therapeutic antiretroviral amount of the ingredients of the present combinations of such co-formulated single pharmaceutical form preferably is administered in a single unit dosage form but, if desired, also multiple unit dosage forms, such as two, three, four, five or even more unit dosage forms may be administered. A physician will be able to determine the exact dosage to be given taking into account the severity of the patient's condition as well as the patient's weight, gender and possibly other parameters such as individual differences in absorption, biodistribution, metabolism and excretion rates for each drug as well as other factors known to those skilled in the art.

This invention also provides a method of treating HIV infected patients said method comprising administering a combination as specified herein.

Furthermore there is provided a method of treating HIV infected patients, said method comprising administering TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor and/or a nucleotide reverse transcriptase inhibitor, in which method a therapeutically effective amount of TMC278 and the nucleoside reverse transcriptase inhibitor and/or nucleotide reverse transcriptase inhibitor can be administered once daily.

Furthermore there is provided a method of treating HIV infected patients, said method comprising administering TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor, in which method a therapeutically effective amount of TMC278 and the nucleoside reverse transcriptase inhibitor can be administered once daily.

In a further aspect of this invention concerns a method of treating HIV infected patients said method comprising administering TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and a nucleotide reverse transcriptase inhibitor, in which method a therapeutically effective amount of TMC278 and the nucleotide reverse transcriptase inhibitor can be administered once daily.

Still a further aspect of this invention comprises a method of treating HIV infected patients said method comprising administering TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor, and a nucleotide reverse transcriptase inhibitor, in which method a therapeutically effective amount of TMC278, the nucleotide reverse transcriptase inhibitor and the nucleoside reverse transcriptase inhibitor can be administered once daily.

Still a further aspect of this invention comprises a method of treating HIV infected patients said method comprising administering TMC278 or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with a nucleoside reverse transcriptase inhibitor, and a second nucleoside reverse transcriptase inhibitor different from the former nucleoside reverse transcriptase inhibitor, in which method a therapeutically effective amount of TMC278, the nucleoside reverse transcriptase inhibitors can be administered once daily.

The active ingredients in the methods of the invention are administered in therapeutically effective amounts, the latter meaning an amount that is sufficient to exert a sufficient HIV inhibitory effect during a certain time period, i.e. the time period between each intake of the formulations, preferably for about 24 hours.

Particular embodiments are methods as specified above wherein one or more of the specific active ingredients mentioned herein such as emtricitabine, racemic FTC, lamivudin, tenofovir and its prodrug tenofovir disoproxil fumarate, are administered.

One embodiment of the present invention relates to the present combinations for use as a medicine. Another embodiment relates to the combinations of the present invention for use in the manufacture of a medicament to treat HIV infected patients.

Of particular interest are any of the combinations as specified herein, or any of the products, pharmaceutical formulations, unit dosage forms, methods and uses being based on said combinations, wherein TMC278 is E-TMC287, or preferably TMC278 hydrochloride salt or more preferably E-TMC278 hydrochloride salt.

The combinations of this invention are especially useful for the treatment of AIDS and related clinical conditions such as AIDS related complex (ARC), progressive generalised lymphadenopathy (PGL) or AIDS related neurological conditions such as multiple sclerosis. The present triple combination may be particularly useful for the treatment of drug-naïve HIV infected patients.

The combinations of the invention are also useful for the prevention of HIV transmission or infection in humans, in particular sexual transmission. Thus, the present invention relates to the use of combinations according to the present invention for the manufacture of a medicament for the prevention of HIV infection or transmission via sexual intercourse or related intimate contact between partners. The invention also relates to a method of preventing HIV infection or transmission via sexual intercourse or related intimate contact between partners comprising administering to a subject in need thereof an effective amount of any of the combinations according to the present invention.

The respective daily dose for each of the active ingredients of a combination according to the present invention may range between 10 mg and 800 mg, preferably between 50 and 400 mg, more preferably between 50 and 300 mg, or between 100 and 300 mg. In particular, the daily dose for TMC278 may range between 10 mg and 500 mg,

preferably between 10 and 300, more preferably between 50 and 250 mg, still more preferably between 50 and 200 mg, e.g. about 100 mg.

The weight ratio of each couple of components of the triple combination taken on a daily basis may vary in a range from 1/10 to 10/1. Suitably, the weight ratio of each couple varies between 1/6 and 6/1, more suitably 1/4 and 4/1, preferably between 1/3 and 3/1, and more preferably between 1/2 and 2/1.

Table 2 lists some examples of the daily dose for each of the active ingredients in combinations of compound E-TMC278, emtricitabine and tenofovir.

Combination no.	E-TMC278	Emtricitabine	Tenofovir
1	50 mg	200 mg	-
2	50 mg	-	300 mg
3	100 mg	200 mg	-
4	100 mg	-	300 mg
5	200 mg	200 mg	-
6	200 mg	-	300 mg
7	50 mg	200 mg	300 mg
8	100 mg	200 mg	300 mg
9	200 mg	200 mg	300 mg

Table 3 lists some examples of the daily dose for each of the active ingredients in combinations of TMC278, abacavir and lamivudine wherein the dose mentioned in the table for abacavir sulfate is the equivalent dose of abacavir base.

Combination no.	E-TMC278	Lamivudine	Abacavir sulfate
1	50 mg	150 mg	300 mg
2	100 mg	150 mg	300 mg
3	200 mg	150 mg	300 mg

Thus, an interesting combination according to the present invention comprises compound E-(A) in a daily dose ranging between 10 mg and 500 mg, a daily dose of 150 mg lamivudine and a daily dose of an equivalent of 300 mg abacavir base. Suitably, such combination is formulated in a single pharmaceutical form.

Another interesting combination according to the present invention comprises compound E-(A) in a daily dose ranging between 50 mg and 250 mg, a daily dose of

150 mg lamivudine and a daily dose of an equivalent of 300 mg abacavir base. Suitably, such combination is formulated in a single pharmaceutical form.

The present invention also relates to a pharmaceutical composition in a form adapted to be applied to a site where sexual intercourse or related intimate contact can take place, such as the genitals, rectum, mouth, hands, lower abdomen, upper thighs, especially the vagina and mouth, comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of a combination according to the present invention. As appropriate special adapted compositions there may be cited all compositions usually employed for being applied to the vagina, rectum, mouth and skin such as for example gels, jellies, creams, ointments, films, sponges, foams, intravaginal rings, cervical caps, suppositories for rectal or vaginal application, vaginal or rectal or buccal tablets, mouthwashes. To prepare such pharmaceutical compositions, an effective amount of each of the particular compounds of the triple combination as the active ingredients is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of administration. In order to increase the residence time of such pharmaceutical composition at the site of administration, it may be advantageous to include in the composition a bioadhesive, in particular a bioadhesive polymer. A bioadhesive may be defined as a material that adheres to a live biological surface such as for example a mucus membrane or skin tissue.

Thus, the present invention also relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of each of the compounds of the present triple combination characterized in that the pharmaceutical composition is bioadhesive to the site of application. Preferably, the site of application is the vagina, rectum, mouth or skin, most preferred is the vagina.

Otten RA *et al* in Journal of Virology (2000), 74(20), 9771-9775 and Witvrouw M *et al* in Antiviral Research (2000), 46(3), 215-221 disclose the ability of tenofovir to delay HIV viral breakthrough after high-risk sexual exposure.

Pani A *et al* in Antiviral Chemistry & Chemotherapy (2001), 12(Suppl. 1), 51-59 describe the ability of lamivudine to delay viral breakthrough.

The ability of TMC278 to prevent HIV infection via sexual intercourse or related intimate contact between partners can be demonstrated in the following test. Immature monocyte derived dendritic cells (immMO-DC) represent a good model for interstitial dendritic cells, which are early targets during sexual HIV transmission and important

initiators of the immune response. These immMO-DC were used in "in vitro" models to test the prevention of HIV infection via sexual intercourse or related intimate contact between partners. One such model is described in the experimental part and indicates that the TMC278 potently inhibits HIV replication in MO-DC/ CD4(+) T cell co-cultures.

Examples

Example 1: Pharmacokinetics of E-TMC278

A double-blind, randomized, placebo-controlled Phase I trial was designed to evaluate safety, tolerability, and *ex-vivo* pharmacokinetics of single doses of compound E-TMC278 in healthy male volunteers. Oral doses of 12.5, 25, and 50 mg were formulated in PEG 400 and taken with a standard meal. The pharmacokinetic results are shown in Table 4.

The pharmacokinetic results of another double-blind, randomized, placebo-controlled Phase I study with 4 dosing sessions to evaluate the safety, tolerability, pharmaco-kinetics and *ex-vivo* pharmacodynamics of single 100 mg and 200 mg oral doses of compound E-TMC278 in healthy male subjects are also reported in Table 4. Randomization was such that for each session 6 subjects received the same dose of compound E-TMC278 and 3 subjects received placebo. There was a time interval of about 14 days between each dosing session

Table 4 shows that high and dose-proportional exposures were obtained. The correlation coefficient for the 5 C_{max} datapoints is 0.9897 and for the area under the curve values between 0 and 48 hours (AUC_{0-48hr}) 0.9952. Half-life of plasma concentrations ranged between 37 and 39 hours. The compound was well tolerated by the volunteers. No relevant adverse effects of the drug were noted.

Table 4

Parameter	12.5 mg	25 mg	50 mg	100 mg	200 mg
C_{max} (ng/ml)	73±14	149±32	267±27	482±121	807 ±207
T_{max} (hr)	4.0±0	4.0±1.3	4.0±1.3	4.3±0.8	4.3 ±0.8
AUC_{0-48hr} (nghr/ml)	1337±310	2805±496	5094±509	8162±2251	15592±2746
$AUC_{0-\infty}$ (nghr/ml)	2210±473	4637±1164	8872±1342	15844±4592	
$T_{1/2}$ (hr)	37.1	38.7	45±9	55±18	

Example 2: Virological profile of Compound E-TMC278

Compound E-TMC278 was tested in a cell-based assay, using natural host cells of HIV. MT-4 cells (a cell line of human T cells) were infected with HIV-1 (wild type or mutants) and exposed to different concentrations of antiviral compound in the presence of 10% fetal calf serum. Cytotoxicity was determined in parallel with the antiviral activity so that the selectivity of the antiviral effect could be assessed. Active compounds have to penetrate the cell membrane in order to interfere with replication steps inside the cell.

After four days of incubation at 37°C, the viability of the HIV and mock-infected cells was assessed by an automated tetrazolium-based colorimetric assay. This method enabled the calculation of both the 50% inhibitory concentration for inhibition of viral cytopathicity (IC50), the IC90, and the 50% cytotoxic concentration (CC50). The ratio CC50/IC50, also called the selectivity index, is an indication of the specificity of the antiviral effect. Tested HIV strains included: Wild type (wt) HIV-1; a panel of single and double mutants, obtained by site-directed mutagenesis (SDM), and a panel of clinical isolates, selected for resistance against NNRTIs.

Activity towards wild type and SDM mutants

A limited panel of HIV-1 mutants was constructed using site-directed mutagenesis (SDM) and homologous recombination techniques. Compound E-TMC278 was tested against an extended panel of single and double mutants known to be resistant against commercially available NNRTIs. Nevirapine (NVP) and efavirenz (EFV) were included as controls.

The results are shown in Table 5 (values presented are IC50 values in nM). For wild type virus, the obtained IC50 was 0.4 nM (0.15 ng/ml) and the IC90 1.3 nM (0.48 ng/ml). The HIV strain with the lowest sensitivity against compound E-TMC278 within this selection was the double mutant 100I+103N, with an IC50 of about 8 nM and an IC90 of about 16 nM.

Table 5

	NVP	EFV	Compound E-TMC278
wild type	81	1.4	0.4
100I	597	35	0.4
101E	547	5	1.6
103N	2,879	28	0.3
106A	2,983	23	0.2
108I	-	2	0.3

	NVP	EFV	Compound E-TMC278
138K	64	1.3	0.4
179D	161	6	0.6
179E	158	5	0.4
181C	10,000	2	1.3
188C	3,764	5	0.1
188H	241	9	0.2
188L	10,000	78	2.0
190A	4,101	8	0.3
190S	10,000	275	0.1
225H	171	2	0.3
227C	1,816	36	2.0
227L	78	0.3	0.3
234I	45	NT	0.3
236L	41	1	0.3
100I+103N	10,000	10,000	7.9
101E+103N	7,033	84	0.5
103N+181I	10,000	37	1.0
227L+106A	10,000	8	1.0

Development of resistance in vitro

NNRTIs are highly selective inhibitors of HIV-1 but their current clinical use is limited by the rapid emergence of NNRTI (cross-) resistance. The rate of resistance emergence against compound E-TMC278 and the first generation NNRTIs nevirapine and efavirenz was compared in vitro.

MT4 cells were infected with wild type HIV-1 at high multiplicity of infection (>1 infectious virus per cell, to maximize the genetic diversity of the virus population) in the presence of various concentrations of compound E-TMC278 (40, 200, 1000 and 5000 x IC50), and were monitored twice a week for virus replication. Emerging virus was collected for pheno- and genotyping. Cultures without evidence of virus replication were further sub-cultivated in the presence of the same concentration of inhibitor for a total duration of 30 days (10 passages).

Resistance to nevirapine emerged within 3-6 days, at all tested concentrations. Breakthrough virus harboured the typical Y181C mutation. The same experiments with efavirenz resulted in the selection of G190E at all concentrations (up to 5μM) within 3 to 7 days. Compound E-TMC278 did not select for resistant virus within 30 days using wild-type virus. If a double resistant mutant K103N+Y181C (IC50 0.8 nM) was used

instead of wild type virus, resistance did emerge at all tested concentrations. Starting from the single mutants Y181C (IC50 1.3nM) or 103N (IC50 0.3nM), virus breakthrough did not occur at 40 and 200 nM, but did occur at 10 nM.

In this experimental setting of high genetic diversity, HIV-1, resistant to first generation NNRTIs, was selected very rapidly. Resistant viruses harboured only one mutation. In contrast, emergence of HIV-1, resistant to compound E-TMC278 was delayed or did not occur.

Cardiovascular and pulmonary safety of compound E-TMC278

Compound E-TMC278 had little or no effect on cardiovascular and pulmonary parameters in vivo at plasma levels covering and exceeding the targeted plasma levels in man and at concentrations in vitro covering or exceeding the anti-viral concentration in vitro.

Example 3: *In vitro* models to test the ability of compound E-TMC278 to prevent HIV infection via sexual intercourse or related intimate contact between partners.

For instance, in one model, monocyte-derived dendritic cells (MO-DC) were infected for 2 hours with the monotropic HIV strain Ba-L at a multiplicity of infection (MOI) of 10^{-3}. After infection, cells were washed 6 times and resuspended in 10% BCS at 400.000 cells/ml. Autologous CD4(+) T cells were purified out of the lymphocyte fraction of the same elutration as the MO-DC and used at a concentration of 2X 10^6 cells/ml ((ratio MO-DC/CD4(+) T : 1/5).

A serial dilution of a compound of formula (I) (test compound) was added to the MO-DC/ CD4(+) T cell co-cultures. Each experiment was done in 96-well plates, in which each cup contained 50µl of MO-DC, 50µl of CD4(+) T cells and 100µl of test compound. Half of the culture medium, with test compound, was refreshed twice weekly. Supernatants were analysed in ELISA after 14 days of culture. To determine antiviral activity, the test compound concentration able to suppress 50% of the viral replication at the end of the primary cultures (EC50) was measured. For compound E-TMC278, the EC50 value was 0.55 nM.

Example 4: formulations

Tablet formulation of the following composition:

Emtricitabine	300 mg
Tenofovir diisoproxyl fumarate	300 mg
E-TMC278 hydrochloride salt	110 mg
HPMC 2910 15 mPa.s	24 mg

Polysorbate 20	6 mg
Crosspolyvidone	18 mg
Lactose monohydrate	43 mg
Magnesium stearate	3 mg
Talcum	6 mg

The active ingredients and lactose are fluidised and sprayed with a solution of HPMC and polysorbate in water (at an equivalent of 120 ml/tablet). Subsequently crosspolyvidone is added, while still being fluidised, followed by magnesium stearate and talcum. The thus obtained granulate is compressed into 13 mm cylindrical tablets using standard compressing equipment.